

A REVIEW: ROLE OF PHOSPHOROUS SOLUBILISING AND SULFUR OXIDIZING BACTERIA IN MINE RECLAMATION

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ABSTRACT

Mining is a necessary evil leads to various types of environmental damages and deterioration of effected soil area. This makes the need of revegetation in the Affected area i.e. reclamation of mining area to make it suitable for further use. There are various methods to improve the physical, chemical and biological properties of soil from which use of biofertilizer is one of the cost effective and pollution free methods. The current review mainly focused on the role of phosphorous solubilizing and sulfur oxidizing bacteria as biofertilizer to regain the soil fertility in mining area.

KEYWORDS: Phosphorous Solubilizing Bacteria, Sulfur Oxidizing Bacteria, Biofertilizer, Bacteria

INTRODUCTION

Mines and Mining are the areas where ores for mining can be found. These may be above the ground or underground and scattered in fair number around gielinor. But mines are considered as necessary evil because mining leads to deterioration of soil quality and water pollution in affected areas. Major sources of erosion, sediment loading of mining sites can include open pit areas, heap and dump leaches, waste rock and overburden piles. A further concern is that, exposed materials from mining operations may contribute sediments with chemical pollutants, primarily heavy metals. Mining operations routinely modify the surrounding landscape by exposing previously undisturbed earth materials (Guide book for evaluating mining project EIA). So, there is need to reduce pollution and relcamation of mining area to make the mining area suitable for further use after mining.

Use of Biofertilizers in Reclamation of Mining Area

Since Mining results in loss of soil fertility, depletion of useful microbial communities, soil erosion and water quality deterioration, therefore there is an urgent need to develop technologies to reclaim these areas for further potential use. There are many methods to improve the physical, chemical and biological properties of soil from which revegetation is most popular and successful method (Sheoran et al., 2010). But this needs a good soil quality which can be improved by application of biofertilizers. Some of the beneficial microorganisms such as *Rhizobium*, *Azotobacter*, Phosphate solubilizing bacteria and blue green algae etc. can be used as biofertilizer in reclamation program. Soil microbes not only fix the nitrogen symbiotically but some microbes like phosphate solubilizing bacteria renders unavailable form of phosphorous to available form of phosphorous to reduce dependence on phosphorous from external sources. The soil microorganism also exhibit great metabolic versatility to make them suitable for growth in nutrient deficient soil. There are several evidence that suggest the prominent revegetation of disturbed sites when the seedlings of planted species are pre-inoculated with microbial inoculants (Adholeya et al., 1997; Bhatia et al., 1998; Aron, 2002; Fay et al., 1999; Franco et

al., 1997; Haselwandter and bowen, 1996; Mishra et al., 1991; Reddell et al., 1999; Setiadi et al., 2002; Sharma et al., 2001; Sharma et al., 1997). Use of biofertilizers may be helpful in the development of a low cost technology to rehabilitate the mining areas and increase their productivity.

WHAT IS THE BIOFERTILIZER?

Biofertilizers are the products supplied with living cells of different types of microorganism having capacity to convert nutritionally important elements from unavailable to available forms through some specific biological processes. These biofertilizers can be applied to seeds or plant surface or soil (Rokhzad et al., 2008). Beneficial microorganisms in biofertilizers have the ability to accelerate and improve plant growth and protect plants from pests and diseases (El-yazeid et al., 2007). The applicability of microbial inoculums is well known from the history which passes from generation to generations of farmers. It was recognized by the process of decomposition of organic residues and agricultural by-products by the culture organisms through different mechanisms and gives healthy harvest of crops (Halim et al., 2009). The microbial inoculants were started in the late 1940's at Malaysia and peaking up in 1970's with the use of *Bradyrhizobium* inoculation on legumes. Government research institute, the Malaysian Rubber Board (MRB) had been conducting research on *Rhizobium* inoculums for leguminous cover crops in the inter rows of young rubber trees in the large plantations.

In general there are six major steps in biofertilizer making. These include choosing active organism, isolation and selection of target organism, selection of carrier material, selection of propagation method, prototype testing and large scale testing. Biofertilizers are generally prepared as powder form of carrier based inoculants consisting of effective microorganisms. This makes biofertilizer handy, effective, stable and suitable for long term storage. Carrier must be properly sterilized by autoclaving or gamma irradiation. Various types of carrier material are available for seed or soil inoculation. If biofertilizer is to be produced in powder form then tapioca flour or peat are the right carrier materials. Organisms that are commonly used as biofertilizers as constituents are nitrogen fixers, potassium solubilizer and phosphorous solubilizer, or with combination of the molds or fungi. Now a day some sulfur and iron oxidizing bacteria are also included as components of biofertilizers. This review mainly focused on phosphatesolubilising and sulfur oxidizing bacteria as biofertilizer and their role on reclamation of mining area.

Phosphate Solubilizing Bacteria

Phosphorus is the key element in many metabolic processes in plants such as photosynthesis, respiration, energy transfer, signaling etc (Khan et al., 2010) and nitrogen fixation in legumes (Saber et al., 2005). Phosphorus is present in soil as inorganic and organic forms, but in the unavailable form of precipitated mineral complexes. These precipitated forms can not be absorbed by the plants (Rengel and Marschner, 2005). Only 0.1 % of the total phosphorus is present in a soluble form i.e. available to the plants (Zhou et al., 1992). The term phosphate fixation explains the reactions that remove available phosphate from the solution into the solid phase of soil (Barber et al., 1995). Phosphate fixation involves two reactions: (1) phosphate sorption on soil surface and (2) phosphate precipitation by free Al^{3+} and Fe^{3+} . So there is need to solubilize this phosphate to make it available for the plants. The phosphate solubilizing bacteria are the promising organism for the same. The natural occurrence of phosphate solubilizing bacteria in soil was first evidence in 1903 (Khan et al., 2007). There are some phosphate solubilizing fungus also present in soil but with less percentage i.e. 0.1 to 0.5 % in comparison to phosphate solubilizing bacterial presence of 1 to 50 % (Chen et al., 2006). The main ectorhizospheric bacteria are *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia have been considered as effective phosphate

solubilizers (Igual et al., 2001). Bacterial genera of *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* are the most powerful PSB (Whitelaw et al., 2000). *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis*, *Bacillus polymyxa*, *Bacillus sircalmous*, *Pseudomonas striata* and *Enterobacter* can be found as the most important bacterial strains (Subbarao et al., 1998; Kucey et al., 1989). The mechanism of phosphate solubilization involves many different reactions mediated by PSB. PSB release metabolites such as organic acids, which chelate the cation bound to phosphate through hydroxyl and carboxyl groups or lowers the pH or compete for the sorption to the soil surface bound (Nahas et al., 1996). This makes the phosphate free from cation and available to the plants (Hilda and Fraga, 2000; Khiari and Parent, 2005). Some PSB can enhance plant growth by enhancing the availability of some trace elements such as iron, zinc etc. (Ngoc et al., 2006), synthesize some enzyme responsible for hormonal modulation in plants, may limit the available iron via siderophore production (Akhtar and Siddiqui, 2009). H^+ excretion originating from NH_4^+ assimilation as proposed by Parks et al. could be an alternative mechanism of Phosphate solubilization. In contrast to expectations, an HPLC analysis of the culture solution of *Pseudomonas* did not show any organic acid even though solubilization happened (Illmer and Schinner, 1995). They also stated that most probable reason of solubilization without acid production is the release of protons accompanying respiration or NH_4^+ assimilation.

This potential of PSBs makes them the main component of Biofertilizers. The reclamation of mining area demands the increase in fertility of the soil of effected area by fulfilling mineral deficiency, which could be completed by using these PSB as biofertilizers. There is evidence of increase in growth performance of *Acacia nilotica*, *Acacia catechu*, *Butea monosperma* and *Pongamia pinnata* by the application of PSB with the combination of blue green algae (Dubey K. et al., 2006). The PSB solubilize the fixed soil phosphorus and applied phosphate resulting in higher crop yields (Gull et al., 2004).

Sulfur Oxidizing Bacteria

Sulfur is also one of the essential element required by plants for optimum growth. Sulfur occurs in a wide variety of organic and inorganic combinations i.e. unavailable to plants. The dominant form of sulfur taken up by plants is sulfate (Vidyalakshmi R. et al., 2009). Sulfur transformations in soil are primarily done by microbial activities such as oxidation, reduction, immobilization and mineralization. The biological transformations of sulfur have been observed by Vernadskii et al. (1927). The sulfur oxidizing bacteria are mainly gram negative bacteria currently distributed as species of *Thiobacillus*, *Thiomicrospira* and *Thiosphaera*, but some heterotrophs such as *Paracoccus*, *Xanthobacter*, *Alcaligenes* and *Pseudomonas* can also show chemolithotrophic growth on inorganic sulfur medium (Kuenen et al., 1982). Based on metabolism, there are two groups of sulfur oxidizing bacteria: The obligate chemolithotrophs, which can only grow when supplied with oxidizable sulfur compounds and heterotrophs that can also use the chemolithoautotrophic mode of growth. The both groups can use CO_2 as carbon source. The obligate chemolithotrophs include *Thiobacillus thioautotrophicus*, *T. neapolitanus*, *T. dinitrificans* (facultative denitrifier), *Thiobacillus thiooxidans* (extreme acidophile), *Thiobacillus ferrooxidans* (acidophilic ferrous iron-oxidiser), *Thiobacillus halophilus* (halophile) and some species of *Thiomicrospira*. The heterotrophs include *Thiobacillus novellus*, *T. acidophilus* (acidophile), *Thiobacillus aquaesulis* (moderate thermophile), *Thiobacillus intermedius*, *Paracoccus denitrificans*, *P. versutus*, *Xanthobacter tagetidis*, *Thiosphaera pantotrophica* and *Thiomicrospira thymus*.

Biological oxidation of hydrogen sulphide to sulphate is one of the major reaction mechanism of sulfur cycle on earth. Other than hydrogen sulphide bacteria have the ability to oxidize sulfur, sulfite, thiosulfate and various polythionates

under alkaline (Sorokin et al., 2001) neutral or acidic conditions (Harrison, 1984). Aerobic sulfur oxidising bacteria belongs to genera like *Acidianus* (Friedrich, 1998), *Acidithiobacillus* (Kelly et al., 2000), *Aquaspirillum* (Friedrich, 1981), *Aquifer* (Humber et al., 1999), *Bacillus* (Arango et al., 1991), *Beggiatoa* (Strohl et al., 1989), *Methylobacterium* (Kelly et al., 1990; De Zwart et al., 1996), *Paracoccus*, *Pseudomonas* (Friedrich et al., 1981), *Starkeya* (Kelly et al., 2000), *Sulfolobus*, *Thermithiobacillus* (Kelly et al., 2000), *Thiobacillus* and *Xanthobacter* (Friedrich et al., 1981) and are mainly mesophilic. Phototrophic anaerobic sulfur oxidizing bacteria mainly belongs to genera like *Allochromatium* (Imhoff et al., 1998), *Chlorobium*, *Rhodobacter*, *Rhodopseudomonas*, *Rhodovulum* and *Thiocapsa* (Brune, 1989). Some bacteria such as *Thiocapsa roseopersicina*, *Allochromatium vinosum* and *Rhodopseudomonas acidophila* (purple non sulfur bacteria) showed growth in dark (Siefert et al. 1979; Kondratieva, 1989).

Though sulfur may be oxidized by many groups of bacteria, but Waksman (1932) pointed out *Thiobacillia* as the most promising and characteristic group of bacteria performing the oxidative part of sulfur transformation in soil. Inoculation of *Thiobacilli* generally increases the rate of sulfur oxidation (Kapoor et al., 1989). This makes sulfur oxidizing bacteria to be used as biofertilizers. Rock biofertilizers from P and K rocks with sulfur inoculated with *Acidithiobacillus* have been applied to different regions of the rain forest zone and in the semiarid region of the Brazilian Northeast, and results shown were excellent.

Combination of Phosphate Solubilizing and Sulfur Oxidizing Bacteria as Biofertilizer Component

Researchers have found the value of sulfur oxidizing bacteria in enhancing the phosphorous availability to the plants when supplied in combination of rock phosphate with sulfur, organic matter, PSB and sulfur oxidizing bacteria (Chen et al., 1996; Vessey et al., 2003). The acidity of the soil, amount of soluble calcium and type of chelating ligands are the basic parameters affecting the phosphorous availability (Chen et al., 1996). The enhanced phosphorous availability in rock phosphate combined with elemental sulfur and *Thiobacillus* (Biospur) has been also observed by Stamford 2002. The growth and oil production of *Raphanus sativus* was significantly increased when rock phosphate with *Thiobacillus* was used as biofertilizer (Khatbirasool, 2011). Parameters affect the phosphorus availability of rock phosphate when used in combination with elemental sulfur include the type of rock phosphate, the ratio of rock phosphate to elemental sulfur and condition of soil and crop (Rajan S., 2002).

CONCLUSIONS

Bio fertilizers can play key role in reclamation of mining area to increase the soil fertility for the revegetation of the mining area. The phosphate solubilizers mixed with organic material, elemental sulfur and sulfur oxidizing bacteria can be proved as a good bio fertilizer for improving soil quality. Furthermore, biofertilizer can be a good replacement of chemical fertilizers in the terms of cost and reduction of environmental pollution.

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